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Protective effect of some food supplement against the toxicity of cyclophosphamide on the brain

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ABSTRACT

The nervous system had been found to be affected by oxidative stress. Natural dietary supplements had been proved that they have antioxidants properties that may protect our bodies against the effects of free radicals. Aphanizomenon flos-aquae (AFA) are a dietary supplement with scientifically demonstrated health-improving effects especially on the nervous system. Cyclophosphamide (CP) is a widely used medication in chemotherapy and can cause oxidative stress. This study was conducted to investigate the role of AFA in preventing cyclophosphamide-induced adverse effects on the brain tissue of CP-treated rats. It is an experimental study carried out in the period from November 2020 to June 2021. It was performed on 30 albino rats with body weights of 280-320 g. The animals were divided into the following three groups. Group 1 (control group), Group 2 (CP group, received a single dose of CP at 100 mg/kg-1 BW intraperitoneally), and Group 3 (CP+ AFA, received orally extract of AFA for 30 days after CP injection). The morphological and histological structures of the brain were compared in the different groups of rats. Paraffin sections were prepared for histological, histochemical, immune-histochemical and morphometric studies. The data were statistically analyzed. Examined sections showed significant cellular injury in group 2 in comparison to the control groups. Group 3 showed marked improvements in the changes that occurred compared to the second group. These results provide evidence that AFA has a protective effect as they reduced the pathological cellular injuries in the cerebral cortex cells induced by cyclophosphamide.

Keywords: Antioxidant, Cyclophosphamide, Rat, Brain, AFA, Food Supplement.

1. INTRODUCTION

AFA (Aphanizomenon flos-aquae) is a filamentous blue-green algal species harvested every summer in Oregon one of the United States. AFA was sold as

a nutritional food supplement for about 20 years as its nutritional benefits of AFA have been admired by many people (Shimizu, 1996; Bruno, 2001). Aphanizomenon flos-aquae (AFA) metabolize directly molecular nitrogen in the air and synthesize several Peptide Groups of Low Molecular Weight. These peptides are considered as neurotransmitters precursors that are used via various brain regions and body to release other substances and influence metabolic functions (Shimizu, 1996; Bruno, 2001). The ability of the brain neurons to manufacture and utilize neurotransmitters is dependent upon consumption of food and then amino acids concentration in the blood (Janssen et al., 1999; Kusaga, 2002; Kusanga et al., 2002; Szabo et al., 2001; Zucchi et al., 2006). Also, AFA had been proved to be a good source of Omega-3 and Omega-6 that support immune system and build neural fibres in the brain (Cunnane et al., 2009). Using up AFA had been found recover several medical conditions such as reducing tumours size, some cases of Alzheimer's disease and protecting against radiation (Belay, 1993; Galli et al., 1999; Ginsberg, 2000; Parthasarathy et al., 2001; Cooke, 2003; Jicha, 2010).

Cyclophosphamide (CP) is a medication that is used as a chemotherapy that stops the growth of malignant cells and suppresses the immune system. It is used for the treatment of various cancers, multiple sclerosis, systemic lupus erythematosus, and other benign tumors. In addition, it is used in the treatment of nephrotic syndrome and following an organ transplant (Cunnane et al., 2009; Singh et al., 2019). It has a serious neurotoxic side effect by production of reactive oxygen species. The neurotoxicity of cyclophosphamide and its metabolites has been well established (Belay, 1993; Galli et al., 1999; Ginsberg, 2000).

Our aim of this work is to study the protective effect of AFA on the brain of albino rats against the cyclophosphamide-induced damaging effects.

2. MATERIALS AND METHODS

CP was purchased from, Frankfurt, Baxter Oncology GmbH, Germany. AFA-Klamath capsules (350 mg) (Company of German Egyptian Pharmaceutical) were dissolved in distilled water. It was given by gastric tube orally. The dose was 94.5 mg/kg /BW/day for 30 days. It was calculated according to the Paget's formula (Paget and Barnes, 1964). This study is an experimental study carried out in the period from November 2020 to June 2021. In this study, 30 healthy, adult, male albino rats (280-320 g) were used. They were obtained from an animal house at the College of Pharmacy, PSA University. They were kept under standard animal housing conditions with access to food and water ad libitum at the Animal Care Facility. The rats were stayed under supervision for approximately 2 weeks before the start of the experiments for adaptation.

The animals were divided into three groups of ten as follows: Group 1: The control group. Group 2, CP group received a single dose of CP (100 mg/kg BW) intraperitoneally. Before starting our study 100 milligrams of CP injected into four rats to ensure significant histological changes in the brain. The dose was chosen based on previous studies (Mattson, 2000). Group 3 (CP+AFA) received AFA extract orally 500 mg/kg per day for 30 days after a cyclophosphamide injection intraperitoneally. All the rats were sacrificed after one month and after dissection and removing the brain, small brain pieces were taken for the histological and histochemical studies. Specimens were prepared for fixation in formalin solution. Then, these sections stained with Harris haematoxylin and eosin (Hx&E) after formation of 5µm thickness sections of Paraffin. After that, some sections stained to detect polysaccharides. For detection of Nissel granules, we used Toluidine blue stain (Bancroft and Gamble, 2008). Programmed cell death and apoptotic changes were detected in the brain of all groups by Caspase-9 immunostaining (Kaufman et al., 1998).

The image analyzer was used to take out the morphometric data. For instance, carbohydrate content of the neuronal cells using PAS-stained and Caspase-9 immnuostained sections were used. PAST 3.0 Version of statistical analyses was performed *via* statistical software (Hammer *et al.*, 2001). The obtained data were expressed as mean • ± standard deviation (SD). The significant level was $p<0.05$.

3. RESULTS

In Group 1, examination of normal brain sections "stained with Hx&E" revealed normal cells of the brain include neuronal, glial, microglia, as well as normal background neuropil. All neurons located in close association with a surrounding network of glial cells (Fig. 1). Likewise, In Group 1 Toluidine blue stain showed normal neuronal cell bodies, normal neurons with darkly stained cell membrane and glial cells. With Periodic Acid Schiff stain, there was a strong PAS +ve interaction in neuronal cell membranes and bodies. In addition, with Caspase-9 immunostaining, there was a mild expression of in neuronal cell bodies and cell membranes (Fig. 2).

In Group 2, Brain rats treated with cyclophosphamide (CP) stained with Hx. & E showed vacuolated and expanding neuronal cell bodies, condensed neurons with narrow diameter, deeply-stained pyknotic nuclei of glial cell and vacuolated neuropil. Also, the same group stained with Toluidine blue showed neuronal cell bodies with faintly stained cytoplasm, faintly stained neurons

and faintly stained glial cells. With PAS stain the group 2 showed weak PAS +ve interaction in neuronal cell membranes as well as cell bodies. On the other hand, with Caspase-9 immunostaining showed marked rising in the Caspase-9 expression of immunostaining in the neuronal cells (Fig. 3, 5 A & B and Table 1).

In Group 2, Brain of treated with AFA extract stained with Hx. & E. showed an effective preventing of degenerative changes as most of brain cells are almost similar to the first group. When the same group stained with Toluidine blue showed marked increase in Toluidine blue stain intensity, showed marked increase in Toluidine blue stain intensity. With Periodic Acid Schiff (PAS) stain showed marked increase in carbohydrates content and, with Caspase-9 immunostaining showed a marked increase in the expression of Caspase-9 (Fig. 4, 5 A & B and Table 1).

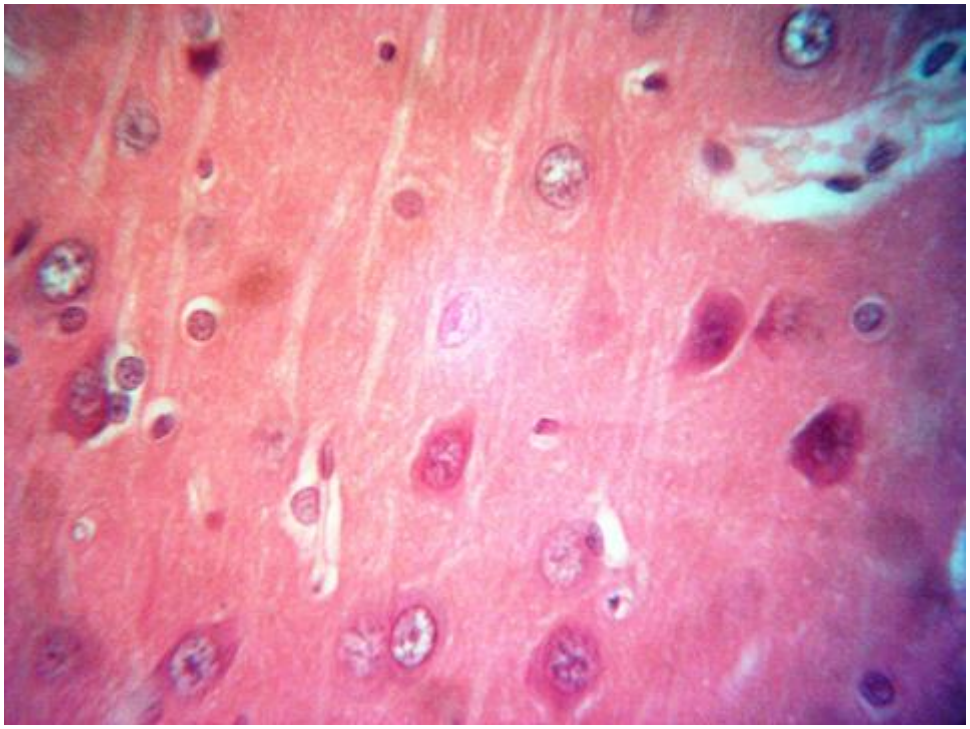


Figure 1 Hx. & E of brain of control group showing normal neuronal and glial cells and also normal a dense network of background (neuropil) (X1000)

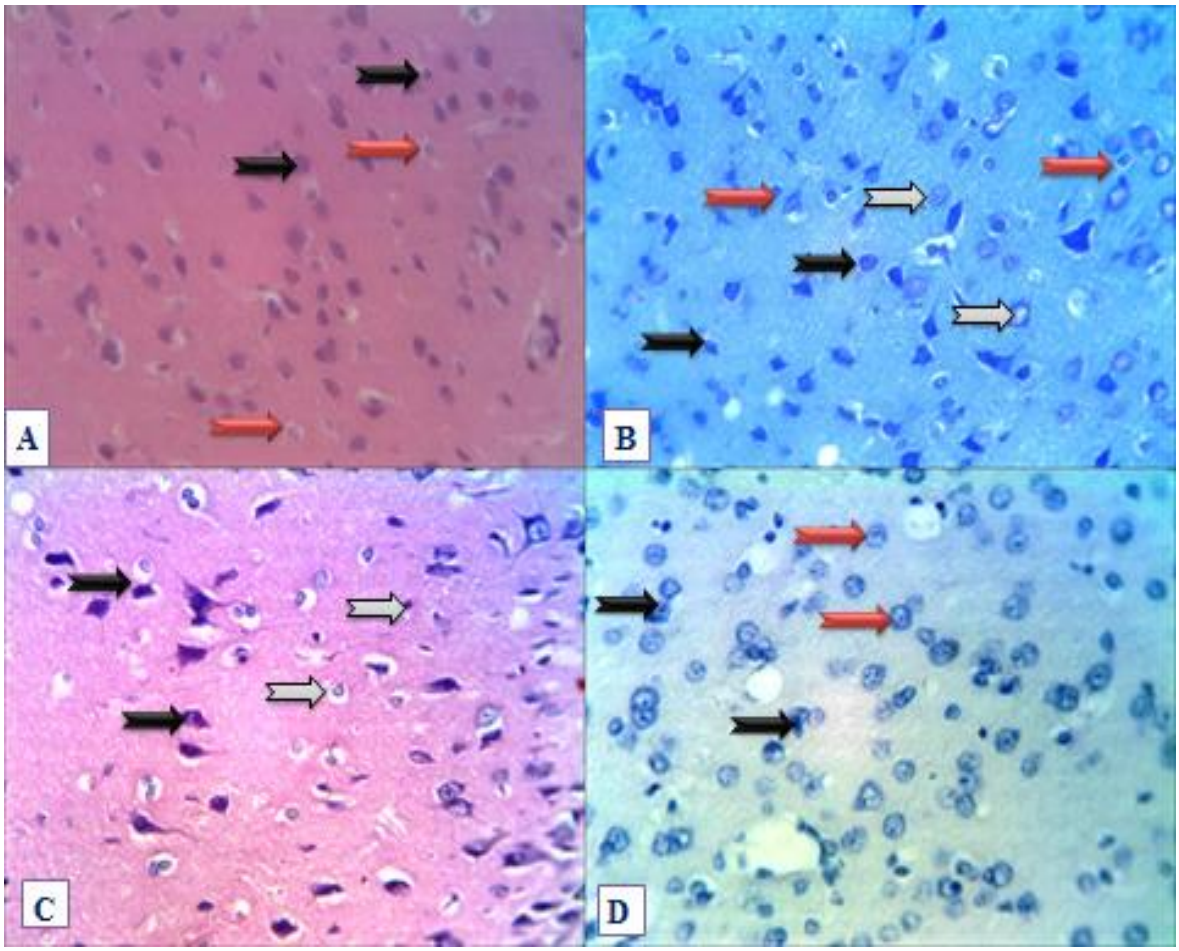


Figure 2 A) Hx. & E of brain of control group showing normal neuronal (black arrows), and glial cells (red arrows), and also normal a dense network of background (neuropil). B) Toluidine blue stain showing normal neuronal cell bodies (black arrows) normal neurons with darkly stained cell membrane (white arrows) darkly stained glial cells (red arrows). C) Periodic Acid Schiff (PAS) with strong PAS +ve reaction in cell bodies (black arrows), as well as in cell membrane of neuron (red arrows). D) Moderate expression of Caspase-9 immuno-reactive in neuronal cell bodies (black arrow) also in cell membranes (red arrows) (X400)

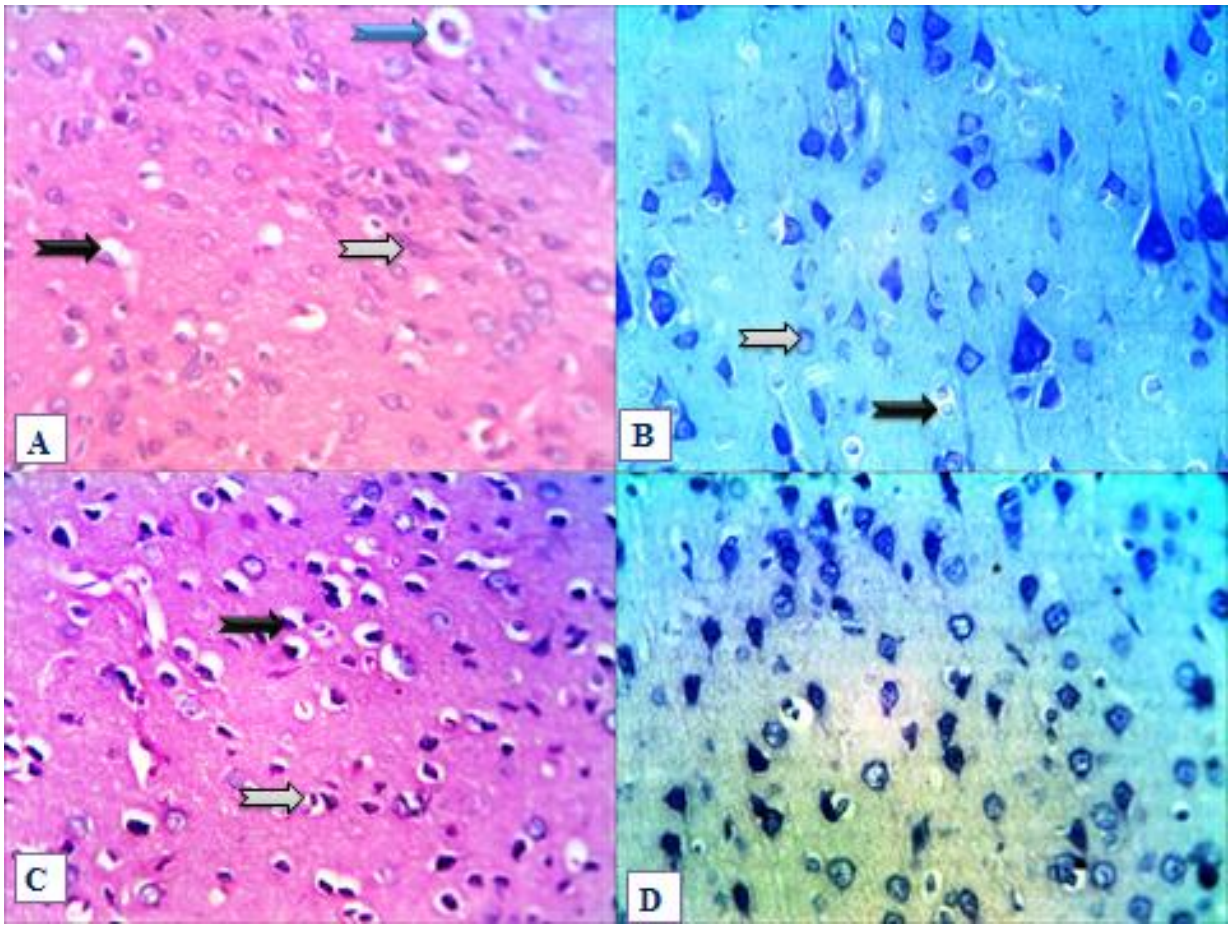


Figure 3 A) Brain rats treated with cyclophosphamide (CP) stained with Hx. & E showing vacuolated and expanding neuronal cell bodies (blue arrow), condensed neurons with narrow diameters (white arrow), deeply-stained pyknotic nuclei of glial cells (blue arrows) and vacuolated neuropil (black arrow). B) Brain rats treated with CP stained with Toluidine blue stain showing neuronal cell bodies with faintly stained cytoplasm (white arrow), faintly stained neurons and faintly stained glial cells (black arrow). C) Brain rats treated with CP stained with PAS showing weak PAS +ve reaction in cell bodies (black arrow), in neurons cellmembranes (white arrows). D) Caspase-9 immunostaining of the brain rats showing marked increase in the of Caspase-9 expression immunostaining in the brain cells (X400)

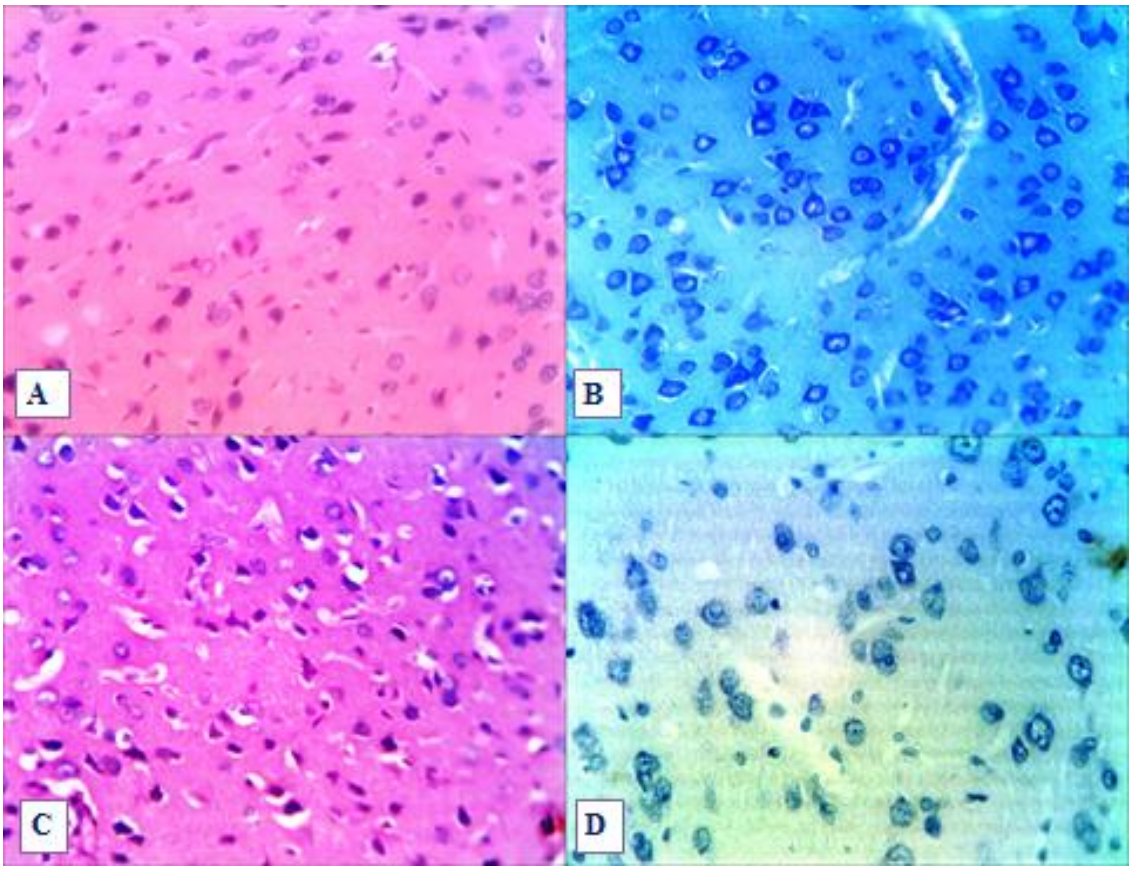


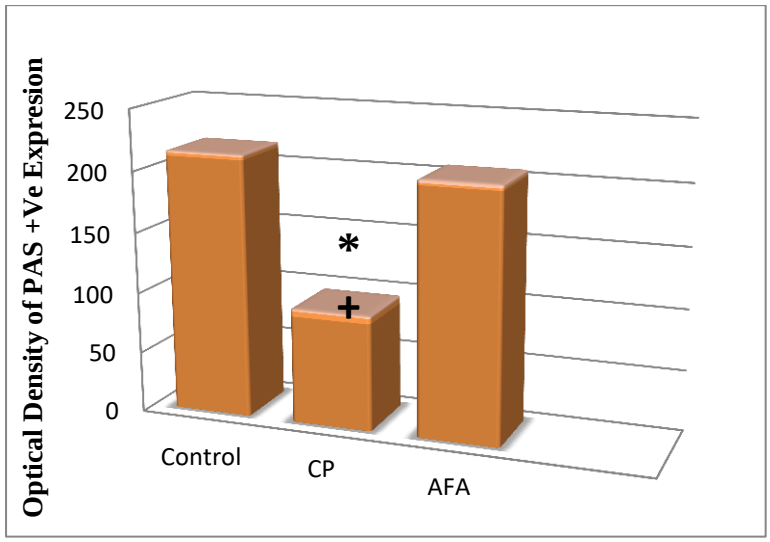
Figure 4 Brain of treated with AFA extract showing effective preventing of degenerative changes as most of brain cells are almost similar to the first group A) Hx. & E. B) Toluidine blue stain. C) Periodic Acid Schiff (PAS) stain showing marked increase in carbohydrates content D) Caspase-9 immunostaining showing marked increase in the expression of Caspase-9 (X400)

Table 1 PAS^{+ve} and Caspase-9^{+ve} expressions Optical Density of the neuronal cells for all groups expressed as mean ± SD.

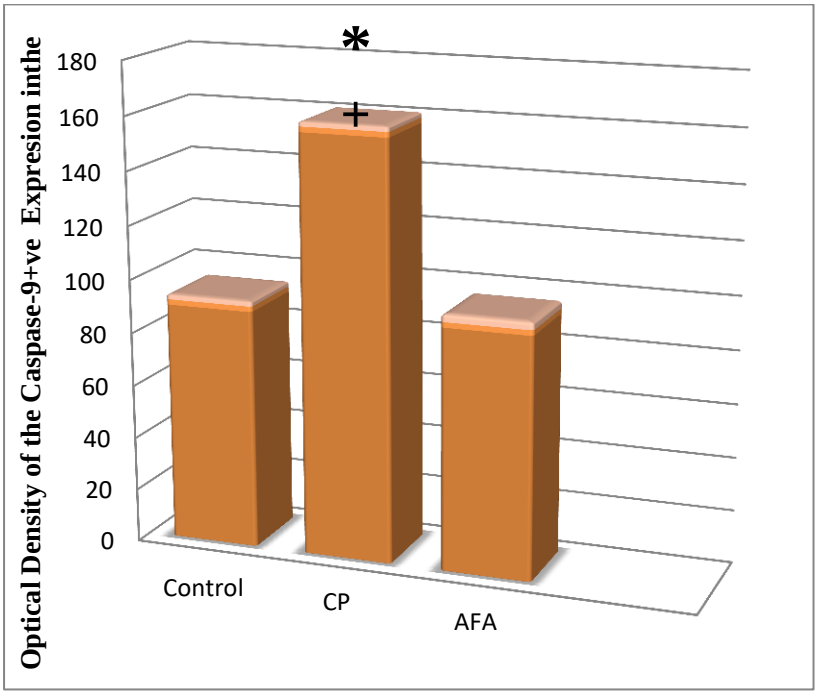
Groups of study	PAS+ve expression of Optical Density in neuronal cells.	Caspase-9+ve expression of Optical Density in neuronal cells.
Control group	211.20 • ± 42.62	89.6 • ± 11.15
CP group	88.40 • ± 13.04+	156.6 • ± 28.67*
AFA group	201.60 • ± 25.61	90.6 • ± 26.48

*Significant increase in the parameters levels compared to the first group (P<0.05).

+Significant decrease in the parameters levels in comparison to the control group.



A



B

Figure 5 A) PAS^{+ve} expression of Optical Density neuronal cells for all groups B) Caspase-9^{+ve} expression in the cerebral cortex neuronal cells

4. DISCUSSION

Brain cells are more at risk from free radicals damage because the brain contains more free form of iron that is responsible for formation of ROS (reactive oxygen species) (Leelavinothan and Munioppan, 2004). Vulnerability of the brain to oxidative stress produced by ROS is due to that it utilizes about one fifth of the total oxygen demand of the body and its relatively poor in antioxidant enzymes content (Vincent, 2008; Seo et al., 2019). It is well documented that dietary antioxidants has a role in protection against the damaging effects of oxidative stress on the cells (Leelavinothan and Munioppan, 2004). Many essential fatty acids like Omega-3 are more frequently found in AFA. Omega-3 usage had been shown to scavenge ROS and free radicals (Liu et al., 1989). Also, the use of Omega-3 had shown a good effect in some nervous diseases in which ROS are the main cause (Leelavinothan and Munioppan, 2004). Moreover, AFA can protect brain through formation of peptides of low molecular weight that are neurotransmitters precursors that start formation of other substances like hormones and affect metabolic functions (Shimizu, 1996; Bruno, 2001).

Some previous studies proved this effect to the increased ROS production accompanied by CNS problems like cerebrovascular hazards, decreased cerebral blood flow and brain oedema (Nasr et al., 2020). In another study on the cerebellum disclosed a weak reaction of PAS, suggesting reduce in the amount of mucopolysaccharides in their cytoplasm (Souza et al., 2010). Moreover, AFA can protect brain through production of low molecular weight peptides which are precursors of neurotransmitters that start secretion of substances and influence metabolic functions (Shimizu, 1996; Bruno, 2001; Helal et al., 2019; Manar, 2013).

Some other studies proved that the antioxidant activity of AFA extract due to the synergic effect of all its various components. The use of natural nutritional supplement could be an important factor for the prevention and treatment of some neurodegenerative diseases. They also found that the AFA extract has a beneficial effect on neurons in which toxicity was induced by neurodegenerative agents (Nuzzo et al., 2018).

5. CONCLUSION

Our study results support these pervious researches as it showed remarkable effects of AFA extract against the degenerative adverse effects of CP on cerebral cortex tissue and cells. Consequently, these results give indicator that AFA supplement may be used as a protective effective method against anticancer drugs as cyclophosphamide.

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Authors' Contributions

All authors contributed to the research and/or preparation of the manuscript. Ali Hassan A. Ali, Abdulrahman M. Alkassar Alanazi and Shaban Ragab Ibrahim participated in the study design and wrote the first draft of the manuscript. Faisal M. Alshmmari, Abduallah A. Alotaibi, Abdulmohsen K. Alghamdi and Shaban Ragab Ibrahim collected and processed the samples. Abdulrahman M. Alkassar Alanazi participated in the study design and performed the statistical analyses. All of the authors read and approved the final manuscript.

Ethics Approval

All series of steps that were implemented in this study that included animal models were in compliance with Ethics Committee of Prince Sattam bin Abdulaziz University Institutional Review Board (PSAU-2020 ANT 4/42PI).

Conflicts of interest

The authors declare that they have no conflict of interest.

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This study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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